H I L G A R D I A

A Journal of Agricultural Science Published by the California Agricultural Experiment Station

VOL. 10 MAY, 1937 No. 14

THE CONNECTION BETWEEN DEMATOPHORA NECATRIX AND ROSELLINIA NECATRIX[1, 2]

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SINCE THE FIRST THOROUGH STUDY of *Dematophora necatrix* (Hart.) by Hartig,[(1)6] who suggested its relationship to the genus *Rosellinia,* there has been a reasonable doubt as to the reality of that relationship in spite of the finding of an associated *Rosellinia* stage by Viala[(6)] and later by Prillieux.[(4)] The reason for this doubt is made clear by Viala, who says:

"Nous avons essayé, par tous les precedes, d'obtinir la germination de ces sporidies sans jamais pouvoir y parvinir. La démonstration expérimentale de la relation des péritheces et des autres formes du *D. necatrix* manque donc."[(6)] (p. 82.)

PRODUCTION OF PERITHECIA

Recently,[(5)] we reported on the occurrence of a highly destructive fungus on apple roots in California that so precisely resembled *Dematophora necatrix,* according to Hartig's description, that we did not hesitate to name it such. From time to time since late 1933, we have collected roots from apple trees killed by this fungus and kept such material in containers under various environmental conditions in an effort to produce the ascigerous stage reported by previous workers. Late in 1935, almost two years to a day after collecting the first material, mature perithecia were observed on four pieces of root that had been kept in moist cham-

[1] Received for publication December 16, 1936.

[2] We wish to express our appreciation to Mr. S. F. Ashby, Director of the Imperial Mycological Institute, and to Mr. E. W. Mason, of the same institution, for sending specimens of *Rosellinia arcuata* and *R. buxi,* and for other courtesies rendered.

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[6] Superscript numbers in parentheses refer to "Literature Cited" at the end of the paper.

bers in the laboratory. In appearance, these perithecia (plate 1, *A, B, E*) are typical of the genus *Rosellinia* and agree closely with the descriptions of those studied by Viala[6] and by Prillieux.[4]

These investigators describe the perithecia as lacking ostioles, whereas in our fungus a definite round pore, or ostiole, is found at the apex of the papilla. The pore is not discernible in old perithecia that have ceased to discharge spores; for in such, the opening is filled with the dried gelatinous material in which the spores are exuded. The structure is readily demonstrated in young perithecia. The ostiole is illustrated by Hartig[2] in *R. quercina,* and we have observed it in *R. aquila* (Fr.) De Not., *R. linderae* Pk., *R. arcuata* Petch., and *R. buxi* H. Fabre.

In spore measurements, also, there is a slight discrepancy: Viala[6] gives the mean size of ascospores as $40 \times 7\mu$, Prillieux the range 43 to $47.5 \times 7\mu$, whereas our measurements, based on 300 spores are: range 31.1 to 47.6×5.1 to 7.1μ, average $37.1 \times 6.3\mu$. We do not, however, consider these differences to be significant. From general observations on size ranges in spores of other species of *Rosellinia,* and for that matter in spores of most fungi, Prillieux's range seems unduly small, which suggests that it was probably based on the measurement of very few spores.

In general, the ascospores are as described by Viala,[6] including the hyaline epispore. This is readily seen in unstained material and very noticeable during early stages of germination, when it often becomes greatly distended just before the germ tubes break through (plate 1, *C*). The spore is typically dorsi-ventral; and in the middle of the ventral side, a slit or suture is seen running parallel to the long axis of the spore and about one-third its length (plate 1, *D*). This slit is not mentioned in the literature as occurring in *Rosellinia necatrix* (Hart.) Berl. nor does Massee[3] mention its presence in *R. radiciperda* Mass. Hartig[2] shows it in *R. quercina* Hart., and we have observed it in the five species of *Rosellinia* examined by us.

GERMINATION OF SPORES, AND PATHOGENICITY

The abundant production of conidia in *Rosellinia necatrix* would lead one to suspect this spore form to be the principal agent of dispersal. Viala[6] reports ready germination of conidia, whereas Hartig[1] made many attempts but succeeded in only one; and in that case the culture was lost before it could be adequately studied. Though we used a large number of media and treated the conidia in various ways to stimulate germination, all our efforts resulted negatively.

At first we had difficulty with the ascospores also, but germination was finally obtained by the following method: Spores were suspended in 2 cc

of 5 per cent lactic acid. After standing for 15 minutes, 10 cc of water was added to reduce the concentration of acid, and the mixture was then poured over the surface of hard potato-dextrose agar (3 per cent agar) in petri dishes at the rate of about 1 cc per dish and incubated at room temperature (22–24° C).

Only about 3 per cent of the spores germinated, and all of those within 24 hours. Subsequent germination tests all gave the same small percentage, with no additional spores germinating after 24 hours. Germination in all cases was through the ventral slit or suture (plate 1, *G*).

Fifty of the germinated spores were transferred singly to potato-dextrose agar, where they continued to grow and eventually (within 24 days) produced the coremial stage of *Dematophora necatrix*. Some of these cultures were used to inoculate eight young apple trees by placing the inoculum in contact with their roots. Within six weeks, all the inoculated trees were dead, whereas the controls remained healthy.

The germination of ascospores, with subsequent production in culture of the coremial stage and the demonstration of pathogenicity, are considered to constitute adequate proof of the genetic relation of *Dematophora necatrix* to *Rosellinia necatrix*. Specimens bearing perithecia have been sent to the Imperial Mycological Institute at Kew and the New York Botanical Gardens.

ASSOCIATED FUNGI

Viala[6] describes in detail a pycnidial fungus which he considers to be a stage in the life cycle of *Rosellinia necatrix*. Massee[3] also describes a pycnidial stage in *R. radiciperda*, and Hartig[2] found pycnidia associated with *R. quercina* but states that he was unable to prove the relationship.

We have found constantly associated with *Rosellinia necatrix*, on apple roots, a pycnidial fungus, which upon culture proved to be a species of the form genus *Phomopsis* and in no way related to the true pathogene. The constancy of its presence, however, might easily lead one astray unless culturing is resorted to.

In apple orchards where the root rot is prevalent, and also in orchards where it has not yet been observed, we find another species of *Rosellinia*, tentatively identified as *R. aquila*. This fungus fruits abundantly on old apple prunings left in the orchards from year to year. It is, however, readily distinguished from *R. necatrix*, even in the field, because of its distinct conidial (*Sporotrichum*) stage. In the laboratory, the marked difference in size and shape of ascospores makes differentiation a routine matter.

LITERATURE CITED

[1] Hartig, Robert.
1883. *Rhizomorpha* (*Dematophora*) *nectatrix* n. sp. Untersuch. Forstbot. Inst. München 3:95–153. 2 pl.

[2] Hartig, Robert.
1880. Der Eichenwurzeltödter. Untersuch. Forstbot. Inst. München 1:1–28. 2 pl.

[3] Massee, George.
1896. Root diseases caused by fungi. Kew Bul. 109:1–5.

[4] Prillieux, Ed.
1902. Les peritheces de *Rosellinia necatrix*. Compt. Rend. Acad. Sci. [Paris] 135:275–78.

[5] Thomas, Harold E., H. N. Hansen, and H. Earl Thomas.
1934. *Dematophora* root rot. Phytopathology 24:1145.

[6] Viala, P.
1891. Monographie du Pourridie. 95 p. 7 pl. Georges Masson, Paris.

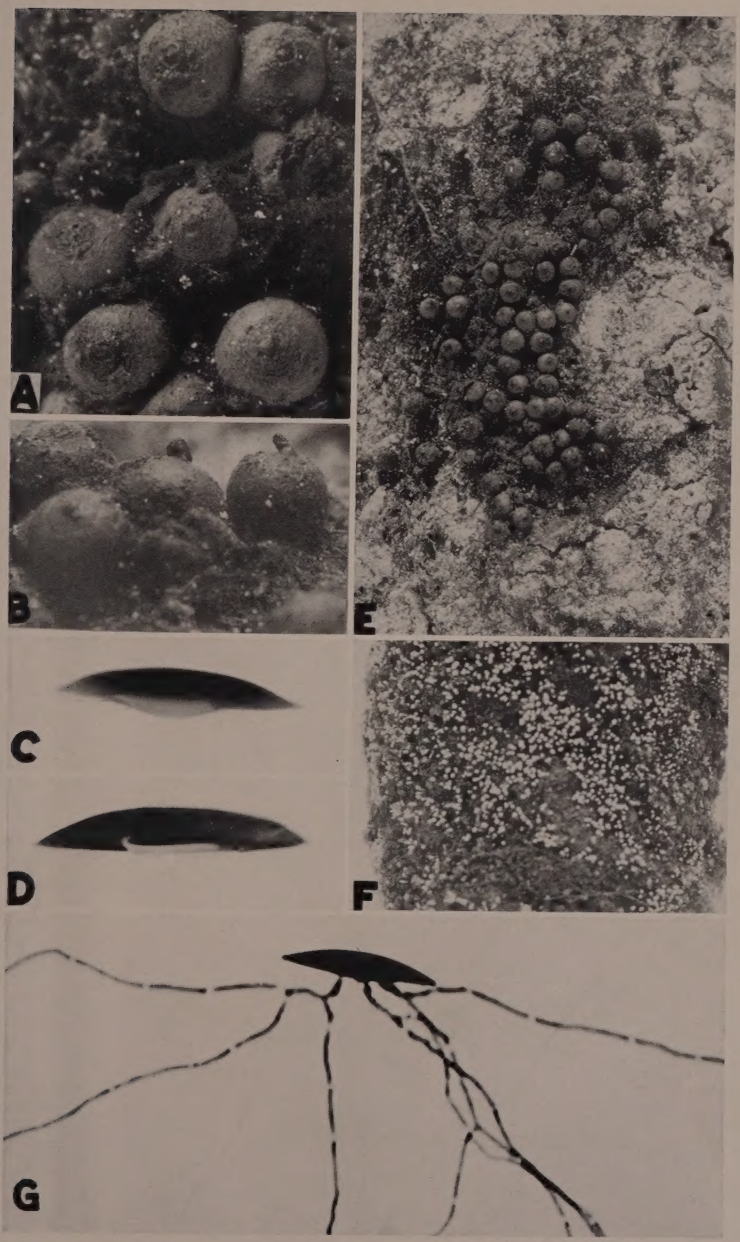

Plate 1.—*Rosellinia necatrix: A* and *B*, perithecia showing freshly exuded spore masses (× 7); *C*, spore, showing the distended epispore just prior to germination (× 900); *D*, spore, showing the ventral slit partly broken (× 900); *E*, perithecia on apple root (× 1.5); *F*, *Dematophora* or coremial stage on apple root (× 1); *G*, germinating ascospore (× 500).

A BACTERIAL GALL DISEASE OF DOUGLAS FIR, PSEUDOTSUGA TAXIFOLIA

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A BACTERIAL GALL DISEASE OF DOUGLAS FIR, PSEUDOTSUGA TAXIFOLIA[1]

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BACTERIAL DISEASES of conifers are exceptionally rare; as far as we know only two have been reported in the literature to occur naturally on twigs, branches, and upper stems of members of the family *Pinaceae*, and both of these are probably produced by the same organism.

In 1888 Vuillemin,[(3)][4] isolated an organism from galls occurring on twigs of *Pinus halepensis* Mill. and named it *Bacterium pini* Vuill. In 1911 Von Tubeuf[(2)] isolated what he considered to be the same organism from galls on twigs and branches of *Pinus cembra* L. Several attempts were made by Vuillemin and by later investigators to produce the disease by inoculating with pure cultures of *B. pini* and by transfer of gall material from diseased to healthy plants, but in no case were positive results obtained.

In 1933 Hansen and Smith[(1)] published a brief note recording the finding of bacterial galls on Douglas fir (*Pseudotsuga taxifolia* Britt.) in California. The present paper reports additional studies of this disease, its transmission, and the pathogene involved.

ECONOMIC IMPORTANCE

The disease has been observed commonly in parts of Napa, Lake, Santa Cruz, Amador, and Siskiyou counties in California, in marginal localities for the growth of Douglas fir, which here occurs in mixed stands composed of several species of conifers and three or four species of broad-leaved trees. These marginal localities, though practically worthless for timber production, are much used for recreational areas, sanitariums, summer resorts, and private summer homes. As far as the value of such places may be materially lowered by the presence of dead, dying, and deformed trees, the disease can be considered to be of economic importance. In its present known range, the Douglas-fir gall disease is otherwise of no economic importance, and of only potential interest to the lumbering industries. Should it invade areas where Douglas fir is now the predominating species, it might very readily become an important

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[4] Superscript numbers in parentheses refer to "Literature Cited" at the end of the paper.

factor in determining the future composition of the forest. If the reproduction of a single member of the stand is attacked, associated species would gain a natural advantage, and they should increase numerically in direct proportion to the havoc wrought by the disease.

DESCRIPTION OF THE DISEASE

The disease is characterized by the occurrence of galls on twigs, branches, and upper stems of the host. New galls are formed only on the younger

Fig. 1.—Douglas-fir trees girdled and partly girdled by bacterial galls. (×1/10.)

trees up to about fifteen years of age, and most frequently on very young trees growing in crowded stands in rather damp situations near streams, ponds, or swamps. The galls most often occur on twigs or small branches but not uncommonly also on the main stems, where they occasionally completely girdle a tree, and thus give rise to an unsightly dead top commonly referred to as "spike top" or, if a secondary leader is formed, it is called a "stag head" (fig. 1). Most of the trees handicapped by having one or more galls on the main stem usually die within a few

years because they are unable to compete successfully with healthy trees. Occasionally an isolated tree with stem galls will live for several years and may even reach small timber size; but since the galls also continue to grow, the trunk is usually so badly deformed that it is useless for anything but firewood (fig. 2). The health of the host does not appear to be

Fig. 2.—Part of Douglas-fir stem, showing old galls. (×1/10.)

seriously threatened by infected twigs since these are gradually shaded out and dropped as the tree grows older.

The galls vary in size from that of a pinhead to several inches in diameter. They are globular in shape, with a rough, spongy, fissured surface which breaks out in a typical more or less cross-shaped pattern (fig. 3, *A* to *D*). During the first year, they are much lighter in color than the bark of the host and therefore stand out rather prominently. In older galls, the shape is materially altered and the typical surface markings gradually disappear. These changes in appearance can probably be attributed to insects and saprophytic fungi, which invade practically all galls that are more than one year old.

Fig. 3.—*A*, Natural galls showing typical cross-shaped markings (×1½); *B*, *C*, and *D*, galls produced by experimental inoculation (×1); *E*, section of natural gall, with arrow indicating point of origin (×1); *F*, twig of Douglas fir from which bark has been stripped to show wounds caused by *Chermes cooleyi* (×1).

The gall is composed of hypertrophied tissues, involving both stele and cortex, and is very similar in structure to that of the olive-tree galls produced by the bacterial pathogene *Bacterium savastanoi* E.F.S. There is this important difference, however, that the olive-tree gall can be produced in cortical tissues without involving any of the xylem elements,

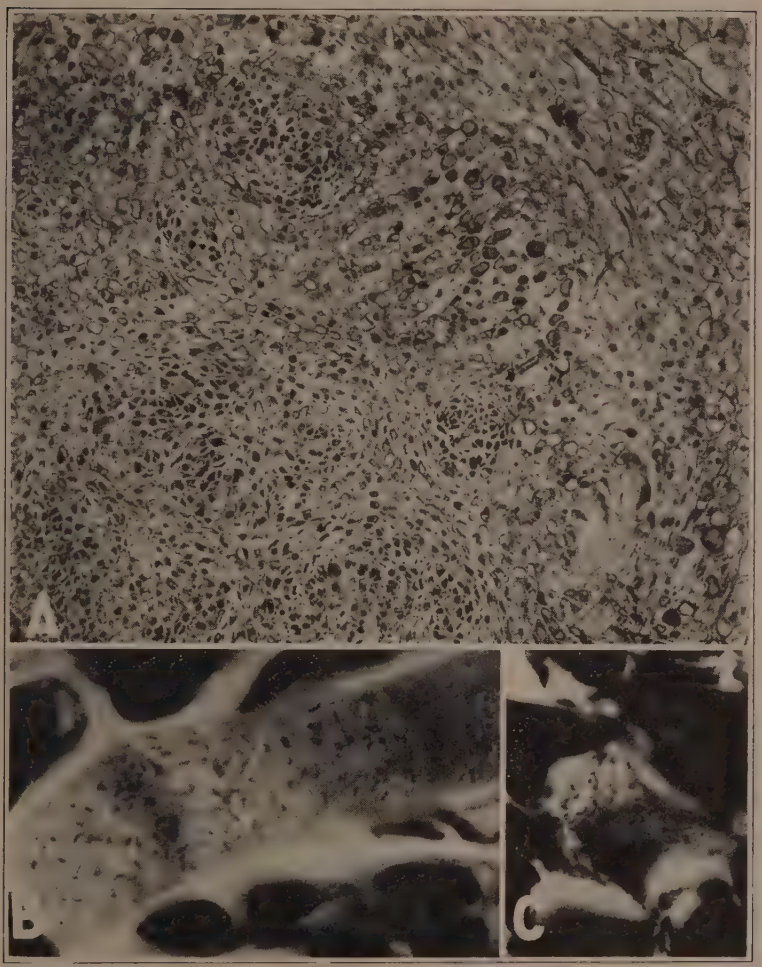

Fig. 4.—*A*, Section of a gall showing groups of rapidly dividing cells and also the presence of woody elements among these groups; *B* and *C*, photomicrograph of *Bacterium pseudotsugae* n. sp. in intercellular spaces.

whereas in all the Douglas-fir galls examined, both those occurring naturally and those produced experimentally, the inner core was found to be always woody in nature, and furthermore the point of origin of the galls was always found to be located within the stele (fig. 3, *E*). The internal structure of the galls is characterized by the presence of many more or less spherical groups of rapidly dividing cells, near or in the

centers of which occur comparatively large intercellular spaces which contain the pathogene (fig. 4, *B* and *C*).

PATHOGENICITY OF THE CAUSAL ORGANISM

Douglas firs were inoculated for the first time on May 15, 1932. Bacteria from pure 48-hour-old cultures were smeared on freshly made leaf scars on previous season's growth and inoculated into the cortex in shallow needle stabs and into the wood in deep needle stabs. Observations were made at various intervals during summer and fall, but all the stab wounds, inoculated and controls, appeared to have healed over rapidly and all looked alike. Additional inoculations by the same methods were made on the fifteenth of January, February, March, and April of 1933.

When the January inoculations were examined on April 15, small galls varying from 1 to nearly 5 mm in diameter were found on the inoculated deep-stab wounds. None of the leaf-scar or shallow-cortex wounds showed any signs of gall formation. Upon examination of the trees inoculated in May of the previous year, it was found that all of the deep-stab inoculations had developed small galls and that inoculated leaf scars and shallow stabs had not.

During the summer of 1933, galls developed on all deep-stab inoculations made in January, February, and March but on only one of those made in April. The remainder of these, however, began to show small galls early in April of 1934. This clearly shows that the pathogene can be present in the host for nearly a year before definite symptoms (galls) begin to appear. It is also fairly evident that gall formation and gall growth is limited to the active growth period of the host, which is roughly from late March to July in Berkeley, California, where the experimental work was done.

The causal organism was reisolated from experimentally produced galls and used for inoculations made in August, 1933, and in January, 1934. Of the 10 wounds inoculated in August, only 3 developed galls. This indicates that the organism finds it relatively difficult to establish itself during late summer, perhaps because of the greatly reduced growth rate of the host at that time of the year. The 8 wounds inoculated with the reisolate in January produced galls during the spring of 1934.

TRANSMISSION

Actual contact of the pathogene with xylem elements of the host tissues appears to be essential to gall formation, on evidence of the facts that the point of origin of all galls examined was found to be located in the

stele and that no galls were produced in inoculated leaf scars or in shallow-cortex wounds. These facts would seem to eliminate water, the principal agent of dispersal of the organism causing olive knot, as a carrier, and indicate that insects capable of producing rather deep wounds were responsible for transmission.

Wherever we have found the Douglas-fir gall disease, two insects—the orchard cicada, *Platypedia areolata* Uhler.; and Cooley's chermes, *Chermes cooleyi* Gill.—have been present in spring and early summer. The cicada oviposits in twigs of the Douglas fir and in doing so causes deep wounds through which splinters of wood protrude. The egg cases remain within the wood and can be found there several years after the wound has healed. More than 200 cicada wounds from one to four years old have been examined, however, and in no case was there any evidence of gall formation.

The other possible carrier, *Chermes cooleyi,* is a sucking insect that feeds on the juices of young Douglas-fir twigs. In these it produces deep feeding punctures that penetrate through the bark and cortex into the wood. Figure 3, *F,* shows a twig from which the bark has been stripped to show the typical wounds produced by the insect. In the middle of each of the transverse lines is a small hole penetrating into the wood to a depth of about a millimeter. In the early spring of 1934, all our experimental trees were heavily infested with these insects, and they were observed to feed on some of the experimentally produced galls. In August of the same year, we found three galls which were definitely traced to wounds produced by *Chermes cooleyi.* Von Tubeuf[2] suggests that a species of *Chermes* is probably responsible for the spread of *Bacterium pini.*

In the localities where the Douglas-fir gall disease occurs, we find the host associated with the following conifers: *Pinus lambertiana* Dougl. (sugar pine), *P. monticola* Don. (silver pine), *P. ponderosa* Dougl. (ponderosa pine), *Libocedrus decurrens* Torr. (incense cedar), and *Abies concolor* Lindl. and Gord. (white fir). We have never found the disease on any of the above species though branches of some of them were occasionally found intermingled with those of infected Douglas fir. As further evidence that the pathogene is highly specific, it was inoculated into the following plants with negative results: *Pinus halepensis* Mill., *P. lambertiana* Dougl., *P. radiata* Don. (Monterey pine), *Tsuga heterophylla* (Raf.) Sarg. (coast hemlock); and into the following herbaceous plants frequently used to test the pathogenicity of the crown-gall organism, *Pseudomonas tumefaciens* Town.: tomato, begonia, beans, and bryophyllum.

TAXONOMY OF THE CAUSAL ORGANISM

On cultural, morphological, and physiological bases, the causal organism appears to be distinct from previously described plant pathogenes and should therefore be considered a new species. Hence, we suggest the name *Bacterium pseudotsugae.*

TECHNICAL DESCRIPTION OF BACTERIUM PSEUDOTSUGAE N. SP.[5]

A nonmotile rod with rounded ends, averaging in size 1.9–3.9 × 0.5–1.5μ; frequently occurring in pairs; non-spore-forming; Gram-negative, non-acid-fast; stains readily with analine dyes; facultative aerobe; liquefies gelatin; slight H_2S produced; nitrates reduced; no acid in milk; no ammonia produced; starch not hydrolyzed; no acid and no gas produced in lactose, sucrose, or glycerine; acid but no gas produced in glucose, levulose, galactose, and maltose. *On nutrient agar slant,* growth scanty, flat, glistening, smooth-surfaced; translucent whitish; medium unchanged. *On potato dextrose agar slant,* growth moderate, slightly spreading, with wavy margin, slightly raised, glistening; surface somewhat contoured; whitish, translucent, becoming brownish with age; medium unchanged. *On potato dextrose pepton agar slant,* growth abundant, spreading, with irregular margin, flat, glistening, becoming dull with age; surface contoured; grayish white; medium unchanged. *On potato cylinder,* growth moderate, spreading, viscid, white becoming brown with age; medium turns brown. *In nutrient broth,* growth slight, no surface growth, clouding slight, no sediment. *In potato dextrose peptone broth,* growth abundant; partial ring formed; clouding strong; sediment fairly abundant; flocculent. *In S. A. B. broth,* growth moderate, no surface growth; clouding slight; sediment scanty-viscid. *In Fermi's solution,* growth moderate, no surface growth; clouding slight to moderate; no sediment. *In Conn's solution,* no growth. *In Uschinsky's solution,* no growth.

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[5] We are indebted to Mr. George Zentmyer for testing the physiological reactions of the pathogene.

SUMMARY

A gall disease of the twigs and stems of Douglas fir (*Pseudotsuga taxifolia*), is described and shown to be of bacterial origin. It is suggested that the causal organism is insect-transmitted, the carrier being probably *Chermes cooleyi*. Three species of pine, one of hemlock, and several herbaceous plants were inoculated with negative results. A technical description is given of the causal organism, which is named *Bacterium pseudotsugae* n. sp.

LITERATURE CITED

[1] Hansen, H. N., and Ralph E. Smith.
1933. A bacterial gall disease of the Douglas fir. Science **77**:628.

[2] Von Tubeuf, C.
1911. Knospenhexenbesen und Zweig Tuberkulose der Zirbelkiefer. Naturw. Ztschr. Forst u. Landw. **9**:25–44.

[3] Vuillemin, Paul.
1888. Sur une bacteriocecidie ou tumeur bacillaire du pin d'Alp. Compt. Rend. Acad. Sci. [Paris] **107**:874–76, 1184–86.

APPLE MOSAIC

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APPLE MOSAIC[1]

H. EARL THOMAS[2]

AN INFECTIOUS VARIEGATION of apple foliage seems to have been recognized by Vibert in France as early as 1835.[3] Earlier reports from the northeastern United States recently reviewed[1][4] indicate that mosaic of apple is general in that area, though seldom if ever destructive. Mottled apple foliage has been noted in the State of Washington,[3, 4] some of which may represent the disease under consideration here. A mosaic type of disease which is probably distinct has been found on an ornamental apple in Kentucky.[10] A report[2] of apple mosaic has appeared from Bulgaria, but the illustrations accompanying it are more characteristic of noninfectious types of chlorosis.

Specimens of mosaic in the variety Ranier were received from Paradise, California, in June, 1932. The trees had been purchased from a nursery in the State of Washington about five years earlier. In August, 1936, a single tree of Smith Cider obtained from a local nursery was found affected in a garden at Berkeley. Some of the affected trees or branches in the orchard at Paradise show, in addition to the direct loss of functional leaf area, a sparseness of foliage and reduction of terminal growth which seems to be chargeable to the mosaic disease.

PLANTS AFFECTED AND SYMPTOMS

Heretofore the disease has apparently been known only on the cultivated apple, *Pyrus malus*. On this plant, the typical symptoms have been amply illustrated[6, 11] (fig. 1). In addition to the symptoms commonly seen on the apple, there occasionally appears a complete chlorosis of the larger veins (vein clearing) while the remainder of the leaf retains the normal form and color.[10] Of particular interest is the tendency of the chlorotic areas to be entirely killed both at Paradise and Berkeley during the summer months of intense sunlight.

In addition to the varieties Ranier, Smith Cider, and Starking, on which natural infection has been seen in California, all of the following developed symptoms when inoculated by grafting: Golden Delicious, Gravenstein, Lady, Tompkins King, White Pearmain, and Yellow New-

[1] Received for publication December 16, 1936.

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[3] Reported by Bradford and Joley.[3] Original not seen by the writer.

[4] Superscript numbers in parentheses refer to "Literature Cited" at the end of this paper.

town. Several other varieties, including Yellow Bellflower are already on record[a] as susceptible.

Since a rather large number of the relatives of the apple are grown in California for fruit or ornament, several experiments were made to determine whether some of these might be susceptible to the mosaic of apple. Inoculations were made by grafting buds, scions, or inarches

Fig. 1.—The two larger leaves at the right (White Astrachan) are affected by mosaic. The three smaller leaves (Esopus Spitzenberg) are taken from a case of genetic variegation.

from affected to healthy potted plants, or by grafting healthy scions on diseased plants. These tests were on a small scale and involved in some cases only one or two plants. The negative results are therefore not always conclusive.

Cotoneaster harroviana became affected after inoculation by inarching, with the production of pale bands and rings in the leaf blade. The symptoms were inconspicuous, but the virus was recovered without apparent loss of virulence by inoculation from this species to apple and rose.

The loquat, *Eriobotrya japonica,* developed strong chlorotic symptoms resembling those on the apple and in addition, in young leaves, a considerable amount of necrosis along the larger veins, resulting in marked distortion of some leaves (fig. 2). This necrosis developed under glass

and was not preceded by any marked chlorosis as is the case with the apple. The virus was recovered from loquat by inoculation to rose.

Three of 5 plants of toyon (*Photinia arbutifolia*) developed symptoms during the year following inoculation. The chlorotic spots which resulted were similar to those on the apple but few in number.

Although it is difficult to obtain a graft union between the rose and

Fig. 2.—Mottling and leaf distortion of loquat produced by inoculation with the apple-mosaic virus.

members of the pome group, definite symptoms were obtained on 5 of 8 plants of Independence Day and Belle of Portugal roses which were inoculated by inarching with affected apple, *Cotoneaster*, or loquat (fig. 3). The virus was recovered from one of these roses by inoculation to apple. The symptoms vary appreciably and may include vein clearing on occasional leaves, but usually approach the type seen on the leaf at left in figure 3. From the type of symptom and the rate of development in the plant, it is inferred that this disease is distinct from the several virus diseases that have been found occurring naturally on the rose in central California.

A single plant of *Sorbus pallescens* inoculated by a scion of affected apple, developed rather strong symptoms resembling those on the apple.

Attempts to cause infection in *Amelanchier alnifolia* (western service berry), *Crataegus douglasii* (western black haw), *Cydonia oblonga*

(quince), *Pyracantha gibbsii yunnanensis* (yunnan fire thorn), *Pyrus communis* (pear—Bartlett variety), and *Sorbus sitchensis* (western mountain ash) have failed, as have also the attempts to recover the virus from inoculated plants of Amelanchier, Crataegus and pear. Recovery was not attempted with the others in this group.

Thus far the discussion has dealt with what is presumed to be a single disease. Other types of chlorosis are not infrequently found on apple

Fig. 3.—Symptoms on Belle of Portugal rose produced by the apple-mosaic virus.

foliage, some of which are not readily separated by symptoms alone from the typical mosaic. Of five such cases encountered without special search in a three-year period, three seemed to be definitely genetic in origin. One of these (fig. 1) was propagated at Berkeley and gave no evidence of transmission by grafting to healthy apple. In another case, a single orchard tree of the Tompkins King variety bore, on leaves scattered generally through the tree, symptoms that were not distinguished from those of the common mosaic. Scions from this tree were grown at Berkeley during the season of 1935 and additional ones in 1936. None of these developed any symptoms except two which were inoculated by inarching with known mosaic apple shoots. Three of the Tompkins King scions, after growing for one season at Berkeley, were top-worked with healthy scions of the susceptible Golden Delicious variety. The latter also remained free of symptoms except one inoculated as above with known apple mosaic. These results indicate that the agent which produces symptoms in the Tompkins King tree is distinct from the common mosaic virus and does not induce resistance to the latter.

A tree of the kaido crab (*Pyrus micromalus*), growing in a garden at Berkeley, bore symptoms in 1933 suggestive of those figured for ornamental crab apple in Kentucky (Valleau,[10] fig. 25). Two seedling loquat trees that were inarched with affected kaido shoots developed mild chlorotic symptoms and in a few leaves fine necrotic lines and rings, distinct in appearance from the symptoms produced in loquat by the virus of the common mosaic. Scions from the original kaido tree were grafted on a White Astrachan tree in 1933 but no symptoms have developed on the latter nor on Golden Delicious, which was later grafted on the same tree (January, 1935). When scions from this Golden Delicious were transferred nine months later to a plant affected by the common apple mosaic, the resulting Delicious foliage bore typical symptoms of the apple mosaic.

DISSEMINATION AND MOVEMENT OF VIRUS IN THE PLANT

Blodgett, who has demonstrated the transmissibility of the disease by grafts (see Orton and Wood[7]) and who has had this disease under observation in orchards of western New York, reports[5] that during a five-year period there was little evidence of natural increase in the number of affected trees.

In the orchard at Paradise, symptoms are confined to the Ranier variety, where 22 of 53 trees were found affected in July, 1935. No new infections were found in May, 1936. None were found in several dozen trees of Delicious in the same orchard or in a block of Golden Delicious trees adjoining this orchard. The affected tree found in Berkeley was planted about fifteen years ago, yet several other trees in the same garden seemed to be entirely free from the disease. No case of spontaneous infection has been seen among the experimental plants.

While the incubation period may be as short as 53 days in apple inoculated by budding, the movement of the virus through the plant is slow, especially in the toyon and rose. When these plants were inoculated in July in one branch each about 6 inches above the base of the branch, the symptoms appeared near or above the point of inoculation the following spring but had not appeared in other branches after more than two years. One such rose plant did show symptoms on all branches after 27 months.[6] These and other observations show that the virus moves more slowly toward the root than upward.

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[5] Blodgett, F. M. Personal letter, November 11, 1935.

[6] With one of the naturally occurring rose mosaics, symptoms have been seen (in rose) below the point of inoculation in less than 40 days from the time of inoculation.

Bearing upon the manner of dissemination are sap inoculations made on three different occasions on young leaves or stems of 12 seedlings of apple, 2 of *Pyrus baccata*, 5 of toyon, and 5 of *Cotoneaster franchetti*. Carborundum[8] was used as an abrasive on all of these. No certain symptoms were seen on any of the plants up to the time of writing, or more than 6 months after the last inoculation.

There is at the time of writing no direct evidence of dissemination by any means except by grafting. It is apparent, however, that, given an efficient natural vector, the disease is capable of affecting a considerable number of species with appreciable damage to certain of them.

HEAT TREATMENT OF AFFECTED SCIONS

One of the most promising methods of removing virus from a plant which is propagated vegetatively seems to be exposure of affected plants or plant parts to high temperatures.[5] The expression of symptoms under varying temperature conditions (not controlled), suggests that the virus of apple mosaic has a relatively low optimum temperature.

TABLE 1

Effect of High Temperatures on the Mosaic Virus in Detached Dormant Apple Shoots

Temperature	Time	Moisture	Number grafted	Number showing symptoms	Injury to scions by heating
° *C*					
36	7 days	Moist sphagnum	8	6	Moderate
36	11 days	Dry air*	5	0†	Severe
45	60 min.	In water	8	8	Slight
50	30 min.	Dry air	13	10	Slight
50	60 min.	Dry air	8	6	Slight

* The basal ends of these pieces were sealed in a vessel containing water. The portions exposed to the water were discarded.

† All of the scions died within 5 weeks.

In a preliminary test in 1935, a few affected shoots of apple were exposed for 15 and 30 minutes to an air temperature of 55° C. Scions were taken from these and grafted on potted seedlings in the greenhouse. Of 3 such plants which made satisfactory growth, 1 failed to develop any symptoms during 1935. This plant did, however, bear mild symptoms toward the end of the summer of 1936. This plant was of the Gravenstein variety, which is less susceptible than some of the others tested. In the meantime in 1936, several experiments were made in which detached dormant apple shoots were exposed to different temperatures and condi-

tions of moisture and for different intervals of time. The shoots were taken from the last preceding growth cycle and were for the most part 4 to 8 millimeters in diameter. Scions from the heated shoots were grafted on potted apple seedlings and were grown in a lath-house at Berkeley.

The details of these tests and the results are summarized in table 1. The failure of certain plants to develop symptoms is probably due to early death or to insufficient growth of the scions rather than to inactivation of the virus. The expression of symptoms here, as in many other virus diseases, is related to vigorous growth of the plant. Since several of the treatments were near the limit of tolerance of the apple tissues, it seems unlikely that heating of scions will be effective in freeing them from the mosaic virus. There was no recognized evidence of attenuation of the virus in these experiments.

SUMMARY

An apple mosaic found in California seems to be identical with the disease known in the eastern United States.

The disease has been transmitted by grafting to *Cotoneaster harroviana, Eriobotrya japonica* (loquat), *Photinia arbutifolia* (toyon), *Rosa* sp. (rose, Belle of Portugal and Independence Day varieties) and *Sorbus pallescens*.

Heating dormant apple shoots in several ways to near the killing point of apple tissues did not inactivate the virus.

LITERATURE CITED

[1] Bradford, F. C., and Lloyd Joley.
1933. Infectious variegation in the apple. Jour. Agr. Research 46:901–8.

[2] Christoff, Alexander.
1935. Mosaikfleckigkeit, Chlorose und Stippenfleckigkeit bei Apfeln Birnen und Quitten. Phytopath. Ztschr. 8:285–96.

[3] Edson, H. A., Paul R. Miller, and Jessie I. Wood.
1935. Diseases of plants in the United States in 1934. U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Reporter Sup. 90:42. (Mimeo.)

[4] Heald, F. D.
1935. Division of Plant Pathology. *In:* Forty-fifth Annual Report. Washington Agr. Exp. Sta. Bul. 325:50–53.

[5] Kunkel, L. O.
1936. Heat treatments for the cure of yellows and other virus diseases of peach. Phytopathology 26:809–30.

[6] Morse, W. J.
1916. Two apple-leaf troubles new to Maine. *In:* Spraying experiments and apple diseases in 1915. Maine Agr. Exp. Sta. Bul. 252:186–90.

[7] Orton, C. R., and Jessie I. Wood.
1924. Diseases of fruit and nut crops in the United States in 1923. U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Reporter Sup. 33:82. (Mimeo.)

[8] Rawlins, T. E., and C. M. Tompkins.
1934. The use of carborundum as an abrasive in plant-virus inoculations. (Abstract.) Phytopathology 24:1147.

[9] Valleau, W. D.
1932. A virus disease of plum and peach. Kentucky Agr. Exp. Sta. Research Bul. 327:89–103.